FORM PTO-1390 (Modified) (REV 11-2000) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER: **RU-0174**

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR): **09/980360**

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/US00/14875	**30 May 2000**	**3 June 1999**

TITLE OF INVENTION

MODIFIED BIODEGRADABLE POLYESTER MICROSPHERES FOR STABILIZING AND IMPROVING THE RELEASE PROFILE OF DRUGS ENCAPSULATED WITHIN THE MICROSPHERES

APPLICANT(S) FOR DO/EO/US

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Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☐ This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include itens (5), (6), (9) and (24) indicated below.
4. ☐ The US has been elected by the expiration of 19 months from the priority date (Article 31).
5. ☒ A copy of the International Application as filed (35 U.S.C. 371 (c) (2))
   a. ☐ is attached hereto (required only if not communicated by the International Bureau).
   b. ☐ has been communicated by the International Bureau.
   c. ☒ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
   a. ☐ is attached hereto.
   b. ☐ has been previously submitted under 35 U.S.C. 154(d)(4).
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3))
   a. ☐ are attached hereto (required only if not communicated by the International Bureau).
   b. ☐ have been communicated by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☒ have not been made and will not be made.
8. ☐ An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)).
10. ☐ An English language translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).
11. ☒ A copy of the International Preliminary Examination Report (PCT/IPEA/409).
12. ☒ A copy of the International Search Report (PCT/ISA/210).

Items 13 to 20 below concern document(s) or information included:

13. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
14. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
15. ☐ A **FIRST** preliminary amendment.
16. ☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
17. ☐ A substitute specification.
18. ☐ A change of power of attorney and/or address letter.
19. ☐ A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821 - 1.825.
20. ☐ A second copy of the published international application under 35 U.S.C. 154(d)(4).
21. ☐ A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).
22. ☒ Certificate of Mailing by Express Mail
23. ☒ Other items or information:

1) Courtesy copy of the International Application; and
2) Return post card.

JC13 Rec'd PCT/PTO 30 NOV 2001

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR)	INTERNATIONAL APPLICATION NO.	ATTORNEY'S DOCKET NUMBER
09/980360	PCT/US00/14875	RU-0174

24. The following fees are submitted:.

BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) :

		CALCULATIONS PTO USE ONLY
☐	Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO	**$1040.00**
☐	International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO	**$890.00**
☐	International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO	**$740.00**
☐	International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4)	**$710.00**
☒	International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4)	**$100.00**

ENTER APPROPRIATE BASIC FEE AMOUNT =	**$100.00**	
Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (e)).	**$0.00**	

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	16 - 20 =	0	x $18.00	**$0.00**	
Independent claims	3 - 3 =	0	x $84.00	**$0.00**	
Multiple Dependent Claims **(check if applicable)**.			☐	**$0.00**	
TOTAL OF ABOVE CALCULATIONS =				**$100.00**	
☒ Applicant claims small entity status. See 37 CFR 1.27). The fees indicated above are reduced by 1/2.				**$50.00**	
SUBTOTAL =				**$50.00**	
Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (f)). +				**$0.00**	
TOTAL NATIONAL FEE =				**$50.00**	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) **(check if applicable).** ☐				**$0.00**	
TOTAL FEES ENCLOSED =				**$50.00**	
				Amount to be: refunded	$
				charged	$

a. ☒ A check in the amount of **$50.00** to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. _____ in the amount of _____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. **50-1619** A duplicate copy of this sheet is enclosed.

d. ☐ Fees are to be charged to a credit card. **WARNING:** Information on this form may become public. **Credit card information should not be included on this form.** Provide credit card information and authorization on PTO-2038.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

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MODIFIED BIODEGRADABLE POLYESTER MICROSPHERES FOR STABILIZING AND IMPROVING THE RELEASE PROFILE OF DRUGS ENCAPSULATED WITHIN THE MICROSPHERES

Background of the Invention

Recently, there has been a great deal of research focused on the development of controlled release dosage forms for drugs comprising peptides and proteins (Langer, R. Science 1990 249:1527-1533). A wide range of protein drugs such as vaccines, enzymes, hormones, and growth factors are now commercially available in large amounts as human therapeutic agents due to the recent advent of recombinant DNA technology. Most of these protein drugs have a short half-life *in vivo* such that a cumbersome multi-dose therapeutic treatment is required and controlled release technology could alleviate such a problem. This is especially true for insulin, since diabetic patients may require several injections of insulin per day to simulate the serum insulin profile of a healthy nondiabetic human (Chien, Y.W. Drug Development and Industrial Pharmacy 1996 22(8):753-789). A variety of degradable and nondegradable polymers have been utilized as matrices to incorporate protein molecules using conventional technologies available for small molecular size drugs.

Thus far, the most reasonable approach developed is to microencapsulate the protein molecules in injectable biodegradable polymer microspheres (Furr, B.J.A. and Hutchinson, F.G. J. Contr. Rel. 1992 21:117-128). As the polymer degrades, the protein diffuses out in a sustained manner through the enlarged pore channels over the desired period of time. These microencapsulated proteins can be self- administered parenterally, i.e. subcutaneously or intramuscularly, via a syringe.

Among the various biodegradable polymers, poly(L-lactic acid) and its copolymers with D-lactic acid or

glycolic acid provide a wide range of degradabilities from months to years depending on their composition and molecular weight (Lewis, D.D. Controlled release of bioactive agents from lactide/glycolide polymer. In M. Chasin and R. Langer (ed.) Biodegradable Polymers as Drug Delivery Systems, Marcel Dekker, New York, 1990, pp:1-41). These polymers are known to be biocompatible and to cause minimal tissue reaction when implanted for long periods of time when used as surgical suture material. Recently an oligopeptide, LH-RH analog, was successfully encapsulated within biodegradable polymeric microspheres for a one-month therapy with a zero order release profile (Ogawa et al. Chem. Pharm. Bull. 1988 36:2576-2588). However, as polymeric carriers for high molecular weight protein drugs, it is still questionable whether they provide a benign microenvironment for the encapsulated protein molecules. During microsphere formulation, the encapsulated protein is often exposed to numerous unfavorable conditions such as organic solvents and high speed vortexing to emulsify the internal aqueous phase. In addition, various molecular deteriorations of the protein such as denaturation, aggregation, chemical degradation and adsorption onto the polymer surface may result from the creation of an acidic environment within the microspheres during polymer degradation. Thus far, there have been few systematic studies on protein stability issues associated with the protein encapsulation within poly(D,L-lactic acid-co-glycolic acid) microspheres.

Instead, most studies have focused on protein release kinetics without examining the stability of encapsulated protein within the microspheres during storage and release (Cohen et al. Pharm Res. 1991 8:713-720; Alonso et al. Pharm. Res. 1993 10:945-953). In many release studies using microspheres, protein release kinetics is often unpredictable and uncontrollable. They commonly exhibit an

initial burst release followed by a very slow release over an extended period, and demonstrate incomplete release profiles at the end despite significant progress of polymer degradation, where aggregation within microspheres results in slow release of the enzyme carbonic anhydrase (Lu, W. and Park, T.G. J. Pharm. Sci. Tech. 1995 49(1):13-19). It has also been reported that degradation profiles of a protein, atriopeptin III, were different in solution and within microspheres (Johnson et al. J. Control Release 1991 17:61-68). Further, human growth hormone has been reported to form dimers at a faster rate within PLGA microspheres as compared to in solution (Cleland et al. Pharm. Res. 1997 14(4):420-425). These studies suggest that the microenvironment in the solid polymer matrix is quite different from the bulk solution. Accordingly, protein stability issues must be taken into account when interpreting release kinetic results.

It has now been found that the low pH microenvironment within a degrading microsphere is one of the major factors responsible for acid-induced degradation of microencapsulated proteins. Further, it has now been demonstrated that inclusion of a basic excipient such as sodium bicarbonate in biodegradable polymer microspheres significantly minimizes the degradation of unreleased protein within the microspheres by maintaining a near-neutral pH environment thereby resulting in an improved release profile.

Summary of the Invention

An object of the present invention is to provide modified biodegradable microspheres for encapsulation of a drug which comprise a biodegradable polymer and a basic excipient.

Another object of the present invention is to provide a method of improving the release profile of a drug encapsulated within a biodegradable microsphere which

comprises incorporating a basic excipient into the biodegradable polymer to form a biodegradable polymer microsphere and encapsulating the drug within the microsphere.

Another object of the present invention is to provide a method of delivering a drug to a patient which comprises encapsulating the drug in a biodegradable polymer microsphere comprising a biodegradable polymer and a basic excipient and administering the encapsulated drug to the patient.

Detailed Description of the Invention

Presently, an immense amount of research is being focused on the development of controlled release dosage forms for parenterally administered protein drugs. One of the most promising approaches to achieve this goal is to microencapsulate the protein drug in injectable biodegradable polymer microspheres. Various biodegradable polymers have been developed. However, much of the research has focused on poly(L-lactic acid) and its copolymers with D-lactic acid or glycolic acid as these polymers provide a wide range of degradabilities and have a history of tissue compatibility. However, these polymers degrade into acidic end products. It has now been determined through intra-microsphere pH estimation studies that the low pH microenvironment from these acidic end products within a degrading microsphere is a major factor leading to drug instability. The present invention relates to modified biodegradable microspheres which minimize the degradation of drugs, and in particular proteins or peptides, encapsulated within the microsphere, by maintaining a near neutral pH environment within the degrading microsphere throughout its *in vivo* lifetime. A stabilization technique has now been established wherein a basic excipient such as sodium bicarbonate is incorporated into a polymer microsphere. This technique results in a

significant reduction in the covalent dimerization of an unreleased protein drug and an improved *in vitro* release profile for the drug.

In initial experiments, porcine insulin encapsulated microspheres prepared from 50:50 DL-PLGA and L-PLA using both a Double-Emulsion-Solvent-Evaporation and Emulsion--Solvent-Evaporation technique were subjected to *in vitro* release studies. The cumulative percent insulin released after a 30-day incubation period from various polyester microsphere formulations is shown in Table 1.

Table 1: Cumulative Release of Porcine Insulin from Polyester Microspheres in 30 Days

Polymer Type	Fabrication Technique	Cumulative % Released (Absolute Error)
50:50 DL-PLGA	Double-Emulsion-Solvent-Evaporation	6.1(1.7)
50:50 DL-PLGA	Emulsion-Solvent-Evaporation	10.4 (2.0)
L-PLA	Double-Emulsion-Solvent-Evaporation	11.0 (1.2)
L-PLA	Emulsion-Solvent Evaporation	28.7 (5.4)

The cumulative % released depicted in Table 1 represents the average value from replicate *in vitro* release studies. As is obvious from Table 1, the *in vitro* release profiles obtained from these studies exhibited an incomplete release of porcine insulin from all microsphere formulations over the time period investigated.

Polyester microspheres degrade via random chain scission of ester linkage in the polymer backbone. The degradation process generates water soluble oligomers that leach out of the microspheres and contribute to a decrease

in medium pH. Accordingly, during the course of the release experiments, the pH of actual release media as well as unbuffered media containing identical amounts of placebo microspheres were monitored as an indicative parameter of polymer hydrolysis. pH-profiles of buffered release media and unbuffered media containing various microsphere formulations during the 30-day incubation period were generated. These pH profiles of *in vitro* release media indicated that the buffered environment maintains a relatively constant physiological pH, and a pH-drop occurs only after 20 days and 25 days of incubation period for release media containing porcine insulin encapsulated 50:50 DL-PLGA and L-PLA microspheres respectively. Examination of the pH-profiles of unbuffered media containing placebo 50:50 DL-PLGA and L-PLA microspheres indicated an initial pH-drop due to the release of residual acid contained in the polymer, followed by a period of stable pH associated with polymer hydration and a final phase of continuous pH-drop due to polymer breakdown. Microspheres fabricated with L-PLA on account of their greater hydrophobicity undergo hydrolysis at a much slower rate compared to 50:50 DL-PLGA. After a 30-day incubation period, the pH of unbuffered media containing L-PLA microspheres dropped to a value of approximately 3.5, while the pH of unbuffered media containing 50:50 DL-PLGA microspheres dropped to approximately 2.7. Thus, a lack of polymer hydration and breakdown is not a contributing factor to the incomplete release of insulin from various microsphere formulations.

In an attempt to identify the cause of incomplete release of insulin from microsphere formulations, the unreleased insulin after a 30-day incubation period was extracted and analyzed by reverse-phase as well as size exclusion HPLC. The results obtained from these studies are shown in Table 2.

TABLE 2: Degradation Profile of Unreleased Porcine Insulin from Polyester Microspheres after a 30-day Incubation Period

Polymer Type	Fabrication Technique	% Unreleased Insulin (Absolute Error)	% A-21 Desamido insulin (Absolute Error)	% Insulin-Related Degradant RRt=1.10 (Absolute Error)	% Insulin-Related Degradant RRt=1.18 (Absolute Error)	% Insulin-Related Degradant RRt=1.23 (Absolute Error)	% Covalent Insulin Dimer (Absolute Error)
50:50 DL-PLGA	Double-Emulsion-Solvent Evaporation	7.7 (0.5)	9.9 (0.2)	ND	4.4 (0.2)	4.7 (0.6)	29.6 (0.3)
50:50 DL-PLGA	Emulsion-Solvent-Evaporation	12.6 (1.5)	9.9 (0.6)	ND	5.0 (1.9)	3.3 (0.5)	29.4 (1.2)
L-PLA	Double-Emulsion-Solvent-Evaporation	14.2 (1.8)	11.7 (0.5)	5.0 (0.1)	ND	ND	20.2 (1.4)
L-PLA	Emulsion-Solvent-Evaporation	8.2 (1.5)	3.5 (0.5)	3.1 (0.5)	ND	ND	10.1 (5.0)

Values shown are the average obtained from replicate *in vitro* release studies.
ND = not detected.

It was determined by reverse-phase HPLC that the unreleased insulin contained in 50:50 DL-PLGA microspheres degraded into at least three distinct degradation products eluting at relative retention times of 1.06, 1.18 and 1.23. The unreleased insulin contained in L-PLA microspheres was found to degrade into at least two distinct degradation products eluting at relative retention times of 1.06 and 1.10. The peak eluting at a relative retention time of 1.06 was confirmed to be A-21 monodesamido insulin by HPLC co-elution with an authentic sample prepared as described in the official monograph for Insulin Human in United States Pharmacoepia 23, Official Monograph for Insulin Human, 809-810 (1995). Acid treatment (pH<2) of insulin has been previously established to yield as many as six transformation products due to the progressive liberation of ammonia from the six amide groups contained in the insulin molecule (Sundby, F. The Journal of Biological Chemistry 1962 237(11):3406-3411). Previous studies have also indicated that the local pH within PLGA microspheres is significantly lower than the medium pH as a result of trapped degradation products (Park et al. Journal of Controlled Release 1995 33:211-222). The peaks eluting at relative retention times of 1.10, 1.18 and 1.23 were thus designated as "Insulin-related degradants" formed by the loss of two or more amide groups from the insulin molecule. The relative percentages of unreleased insulin and insulin-related degradants formed within the microspheres were estimated based upon the total amount of insulin initially contained in the microsphere release samples by comparison of their peak area responses to a calibration curve of porcine insulin based on the assumption that deamidation does involve a chromophoric change. Size exclusion HPLC analyses of the unreleased insulin from various microsphere formulations indicated that a significant portion of the unreleased insulin had already undergone covalent

dimerization within the microspheres prior to release. The percentage of covalent dimer formed was estimated by Area Normalization technique based on the assumption that the molar absorptivity of the dimer is equivalent to two monomeric units. Comparison of the data presented in Tables 1 and 2 indicates a correlation between the cumulative percent insulin released in 30 days from various microsphere formulations and the extent of covalent dimer formed within these microspheres prior to release. In other words, the data suggest that a higher cumulative percent release of 28.7% from L-PLA microspheres fabricated using Emulsion-Solvent-Evaporation was in turn associated with a reduction in covalent dimerization (10. 1%) of the unreleased insulin.

Since the low pH microenvironment within a microsphere undergoing hydrolysis was believed to be one of the major factors leading to protein degradation, further studies were designed to estimate the intra-microsphere pH-profile during the course of polymer hydrolysis. In order to estimate the gradual drop in intra-microsphere pH, acid-base indicators covering a wide pH transition range were encapsulated in 50:50 DL-PLGA microspheres and the microspheres were subjected to accelerated stability studies at 40°C and 75% relative humidity. The dye loaded microspheres were periodically inspected for color change caused due to the gradual drop in intramicrosphere pH as a result of polymer hydrolysis. For each indicator-encapsulated microsphere, the exact time point during the accelerated stability studies at which, the color of the alkaline form of the indicator was no longer visually perceptible (i.e., the color of the encapsulated indicator was visually perceptible as the acid color), was recorded.

A method to correlate this visual perception of acid color for each indicator encapsulated microsphere with a pH value was therefore devised. This was done by constructing

a pH-scale with an interval of 0.2 pH units using Standard USP buffer solutions of known pH values. The gradual color change of each indicator was visually inspected in a series of standard buffer solutions covering its pH transition interval, and the pH values at which the color of each indicator was visually perceptible to have completely transitioned to the acid color was recorded. These pH values were then correlated with the stability time points at which the corresponding indicators were visually perceived to have completely changed to the acid color within the microspheres. The intra-microsphere pH values estimated at various stability time points using this technique are tabulated in Table 3.

Table 3: Estimation of Intra-Microsphere pH-Profile during Hydrolysis of Indicator Loaded 50:50 DL PLGA Microspheres

Indicator	pH Transition Interval	Acid Color	Alkaline Color	Stability Time Point Corresponding to Acid Color (Hours)	Estimated Intra-Microsphere pH
Bromothymol Blue	6.0-7.6	Yellow	Blue	Initial (upon encapsulation)	6.0
Bromocresol Purple	5.2-6.8	Yellow	Purple	168	5.2
Bromocresol Green	3.8-5.4	Yellow	Blue	432	3.8
Bromophenol Blue	3.0-4.6	Yellow	Purple	576	3.0
Orange IV	1.4-2.8	Red	Yellow	696	1.8
Crystal Violet	0.0-1.8	Yellow	Violet	Failed to change to acid color after 1176 hours	Cannot be Estimated

An intra-microsphere pH-profile as a function of time under accelerated stability conditions was generated and showed that the intra-microsphere pH drops linearly (r^2=0.999) until 576 hours followed by a steep drop in pH, probably as a result of adequate hydration of the polymer leading to its quick breakdown. The photomicrograph of 696-hour stability sample of Orange IV encapsulated microspheres also indicated significant moisture uptake by the polymer. It was estimated from these studies that the pH environment within a microsphere exposed to accelerated stability conditions drops to a value of approximately 1.8 after 4 weeks. These studies thus confirm that the low pH environment within the microspheres was one of the major factors responsible for the degradation of the encapsulated insulin. Since deamidation as well as covalent dimer formation proceed through a common cyclic anhydride intermediate, it was further believed that the low pH microenvironment coupled with the high concentration of trapped insulin within a microsphere undergoing hydrolysis promotes covalent dimerization of the unreleased insulin thereby leading to incomplete release profiles.

Experiments were designed to determine whether a basic excipient such as sodium bicarbonate incorporated in a microsphere formulation would be capable of maintaining a near neutral pH environment by neutralizing the acids released during polymer hydrolysis. Since a high pH environment (>8) would result in a rapid increase in insulin degradation, the amount of base incorporated must be barely sufficient to neutralize acids released during polymer hydrolysis without causing an increase in the intra-microsphere pH. Accordingly, microspheres were fabricated using Emulsion-Solvent-Evaporation technique which employs both insulin as well as sodium bicarbonate in their crystalline form. This method enabled the sodium bicarbonate crystals embedded in the polymer to gradually

solubilize as the polymer underwent hydrolysis, thereby neutralizing the released acids. These microspheres were then subjected to *in vitro* release studies. The *in vitro* release kinetics of porcine insulin were significantly higher from microspheres containing sodium bicarbonate compared to microspheres prepared using identical fabrication technique excluding sodium bicarbonate. Specifically, cumulative percent insulin released in 30 days for 50:50 DL-PLGA microspheres containing sodium bicarbonate was 47.3% compared to 10.4 % for microspheres containing no sodium bicarbonate. After a 30-day incubation period, the release study was terminated and the unreleased insulin was extracted and analyzed by reverse-phase HPLC and size exclusion HPLC.

Size exclusion HPLC analyses of the unreleased insulin from microspheres containing sodium bicarbonate indicated a significant reduction in covalent dimer formation compared to microspheres prepared excluding sodium bicarbonate. In fact, the inclusion of a basic excipient such as sodium bicarbonate as an additive in 50:50 DL-PLGA microspheres almost prevented the formation of covalent insulin dimer to only trace levels that could not be reliably quantitated. The total degradation into deamidated products was also reduced to only 7.1 % in the microsphere formulation containing sodium bicarbonate.

Accordingly, the present invention relates to modified biodegradable microspheres for encapsulation of a drug which comprise a biodegradable polymer and a basic excipient. As demonstrated herein, the modified biodegradable microspheres are useful in stabilizing the encapsulated drug and in improving the release kinetics of the drug. Thus, the present invention also relates to a method of improving the release profile of a drug encapsulated within a biodegradable microsphere which comprises incorporating a basic excipient into the

biodegradable polymer matrix which encapsulates the drug and forms the microsphere. These modified biodegradable microspheres are particularly useful for protein or peptide drugs wherein controlled release formulations are especially desirable. In a preferred embodiment, the biodegradable polymer used in the present invention comprises poly(L-lactic acid) or one of its copolymers with D-lactic acid or glycolic acid. Pharmaceutically acceptable basic exipients for parenteral administration which can be incorporated into the polymer matrix for use in the present invention are well known in the art. Examples include, but are not limited, bicarbonates such as sodium bicarbonate and phosphate buffered saline. The amount of basic excipient to be incorporated into the polymer can be determined routinely by one of skill in the art based upon the ability of the excipient to neutralize acids released during polymer hydrolysis without causing an increase in the intra-microsphere pH.

Also provided in the present invention is a method of delivering a drug, preferably a protein or peptide drug to a patient. In this method, the drug is first encapsulated in a biodegradable polymer microsphere comprising a biodegradable polymer and a basic excipient. Methods for encapsulating drugs, and in particular proteins, are well known in the art. Examples of well known encapsulation techniques include, but are not limited to, the Double-Emulsion-Solvent-Evaporation and Emulsion-Solvent-Evaporation techniques described herein, low-temperature phase separation, emulsion phase separation, prilling and spray drying. The encapsulated drug is then administered to the patient preferably via a parenteral route such as intravenously, intramuscularly or subcutaneously. The following nonlimiting examples are provided to further illustrate the present invention.

EXAMPLES

Example 1: Materials

Poly(D,L-lactic acid-co-glycolic acid) 50:50 inherent viscosity approximately 0.5 (RESOMER RG504) and Poly(L-lactic acid) molecular weight 2000 (RESOMER L104) were obtained from Boehringer Ingelheim Chemicals Inc. (Montvale, NJ). Polyvinyl alcohol, average molecular weight 30,000-70,000 was obtained from Sigma Chemical Company (St. Louis, MO). Crystalline porcine insulin was obtained from Eli Lilly and Company (Indianapolis, IN). All other buffering agents and chemicals used were reagent grade. All solvents used for analysis were high-performance liquid chromatography (HPLC) grade and distilled water was purified to the 18 megaohm resistivity level by filtering through a Millipore Milli-Q water filtration system.

Example 2: Microsphere Preparation

Porcine insulin encapsulated 50:50 DL-PLGA and L-PLA microspheres were prepared using two different techniques, namely Double-Emulsion-Solvent Evaporation as described by Soriano et al. International Journal of Pharmaceutics 1996 142:135-142 and Emulsion-Solvent-Evaporation as described by Kwong et al. J. Control Release 1986 4:47-62.

In the Double-Emulsion-Solvent-Evaporation Method: porcine insulin (20 ± 2 mg) was accurately weighed and dissolved in 100 μl of 30 % aqueous glacial acetic acid solution. About 600 ± 20 mg of the polymer 50:50 DL-PLGA or L-PLA was accurately weighed and dissolved in either 3 ml or 1 ml of methylene chloride respectively depending on the polymer being used. The insulin solution was then slowly poured into the polymer solution dropwise and the resulting mixture was vortexed for 2 minutes using a touch mixer to form the first inner emulsion (w_1/o). The first emulsion was then poured, to 200 ml of a rapidly stirred aqueous solution of 1 % Polyvinyl alcohol to form the second emulsion ($w_1/o/w_2$). The emulsion was continuously stirred

using a plate stirrer for 2 hours to allow the methylene chloride to evaporate. The microspheres were collected by decanting the supernatant and dried in an evacuated dessicator in the presence of phosphorus pentoxide. The dried microspheres were then sieved through a 590 P opening sieve and weighed to determine the yield. The decanted supernatant was assayed to determine the amount of unentrapped insulin.

In the Emulsion-Solvent-Evaporation Method about 600 ± 20 mg of the polymer 50:50 DLPLGA or L-PLA was accurately weighed and dissolved in either 6 ml or 1 ml of methylene chloride respectively depending on the polymer being used. About 20 ± 2 mg of porcine insulin crystals was accurately weighed and suspended in the polymer solution. The suspension was vortexed for 2 minutes using a touch mixer to form a homogenous suspension of insulin crystals in the polymer solution. The resulting suspension was then added to 200 ml of a rapidly stirred aqueous solution of 2 % Polyvinyl alcohol to form (o/w) emulsion and continued in a similar fashion as the Double-Emulsion-Solvent Evaporation method.

Example 3: *In Vitro* Release Study

Insulin release was measured by placing 20 ± 0.5 mg of microspheres in microcentrifuge tubes containing 1 ml of release medium (isotonic phosphate buffered saline, pH 7.4 containing 0.02 % sodium azide as a bacteriostatic agent and 0.001 % TWEEN-85 as a surfactant to prevent the microspheres from forming clumps). The tubes were placed in a shaking water bath at 37°C at a speed of 45 rpm. The tubes were centrifuged at periodic time intervals and 200 μl aliquots were withdrawn and replaced with fresh medium. The insulin released was analyzed by reverse phase HPLC. The pH of the release medium was periodically monitored during the entire course of the release study. In addition, the pH of unbuffered medium containing an

identical amount of placebo microspheres was also monitored at periodic intervals and used as an indicative parameter of polymer hydrolysis. At the conclusion of the release study, the unreleased insulin within the microspheres was extracted by dissolving the polymer in 400 µl of acetonitrile and subsequently extracting the encapsulated insulin in the buffer component of the highperformance liquid chromatography mobile phase (0.25 N phosphoric acid adjusted to pH 2.4 with triethylamine) used for reverse-phase analyses. The samples were then analyzed by reverse-phase as well as size exclusion chromatography.

Example 4: HPLC Analyses

Insulin and related substances were analyzed using a reverse phase gradient HPLC method employing a C-18 Symmetry column, 5 µm, 100 Å, 150 X 3.9 mm (Waters Corporation, Milford, MA). The mobile phase consisted of Acetonitrile:0.25 N Phosphoric acid, pH adjusted to 2.4 with Triethyl amine. A gradient from 22% to 30% Acetonitrile in 25 minutes at a flow rate of 1 ml/minute was used. The detector was set at a wavelength of 210 nm and an injection volume of 20 µl was employed. The related substances peak areas were compared against a calibration curve of insulin standards.

A size exclusion HPLC method consisting of an Insulin HMWP column, 7.8 X 300 mm (Waters Corporation, Milford, MA) was used to determine the percentage of covalent insulin dimer and high molecular weight transformation products. The mobile phase consisted of 0.1% L-Arginine in water:Glacial acetic acid:Acetonitrile (65:15:20) at a flow rate of 0.5 ml/minute. The detector was set at a wavelength of 275 nm and an injection volume of 100 µl was employed.

Example 5: Intra-Microsphere pH Estimation Study

The gradual pH-drop inside degrading 50:50 DL-PLGA microspheres was estimated by encapsulating acid-base

indicators covering a wide range of pH-transition intervals to serve as pH-indicating probes.

50:50 DL-PLGA microspheres encapsulating a wide range of acid-base indicators (Bromothymol Blue, Bromocresol Purple, Bromocresol Green, Bromophenol Blue, Orange IV and Crystal Violet) at a theoretical loading level of -0.7 % were prepared using the Double-Emulsion-Solvent-Evaporation method. Specifically, about 4 ± 0.5 mg of acid-base indicator was accurately weighed and dissolved in 100 μl of water. Indicators which were sparingly water-soluble (Bromothymol Blue, Bromocresol Purple, Bromocresol Green) were first dissolved in 50 μl of 0.01 N sodium hydroxide followed by the addition of 50 μl of water. About 600 ± 20 mg of 50:50 DL-PLGA was accurately weighed and dissolved in 3 ml of methylene chloride. The indicator solution was then slowly poured into the polymer solution dropwise and the resulting mixture was vortexed for 2 minutes using a touch mixer to form the first inner emulsion (w_1/o). Microspheres were then fabricated in an identical manner as described in Example 2.

Studies were designed to estimate the intra-microsphere pH at various time-points during the course of polymer hydrolysis by subjecting a range of acid-base indicator encapsulated microspheres to accelerated stability conditions. i.e., 40°C and 75% relative humidity. The dye loaded microspheres were then periodically inspected visually for color change and for each indicator-encapsulated microsphere, the exact time point during the accelerated stability studies at which, the color of the alkaline form of the indicator was no longer visually perceptible (i.e., the color of the encapsulated indicator was visually perceptible as the acid color), was recorded. The visually observed gradual color change of the indicator encapsulated microspheres at various stability time points was confirmed by placing a specimen of the microsphere

sample on a glass slide and observing under a stereoscopic microscope. The microspheres were photographed with the attached polaroid camera using Polacolor ER Instant Films.

A pH-scale was devised by preparing a series of Standard USP Buffers (Hydrochloric Acid Buffer, Acid Phthalate Buffer, Neutralized Phthalate Buffer and Phosphate Buffer as described in United States Pharmacoepia 23, General Chapters, Composition of Standard Buffer Solutions, pp. 2049-2050 (1995)) of known pH values at intervals of 0.2 pH units. The actual pH values of these buffers were measured and recorded. For each indicator included in the study, a series of buffers at intervals of 0.2 pH units covering the pH transition interval of the indicator under investigation was chosen. A 0.1% aqueous solution of each indicator was added to the corresponding standard buffer solutions covering its pH transition interval, and the color change was visually inspected. The pH values of the buffers at which the color of each indicator could be visually perceptible to have transitioned completely to the acid color was recorded. Intra-microsphere pH at various stability time points was then estimated by correlating the pH values of standard buffer solutions at which point each indicator completely transitioned to the acid color with the stability time point at which only acid color was visually perceptible within the microspheres based on the assumption that two solutions of the same color tone (in this case, acid color) have equal pH.

Example 6: Preparation of Porcine Insulin Microspheres Containing Sodium Bicarbonate

Porcine insulin microspheres with a theoretical insulin loading of approximately 3.2% containing sodium bicarbonate (theoretical loading level of 7.7%) were prepared using the Emulsion-Solvent-Evaporation method. In this method about 600 ± 20 mg of the 50:50 DL-PLGA was

accurately weighed and dissolved in 6 ml of methylene chloride. About 20 ± 2 mg of crystalline porcine insulin and 52 ± 5 mg of sodium bicarbonate were accurately weighed, powdered and mixed to form a homogenous mixture. The mixture was suspended in the polymer solution and vortexed for 2 minutes using a touch mixer to form a homogenous suspension. Microspheres were then fabricated in an identical manner as described in Example 2. The loading level of sodium bicarbonate was based upon the maximum solid that could be physically suspended in the polymer solution to still render it adequately pourable.

What is Claimed is:

1. A modified biodegradable microsphere for encapsulation of a drug which comprises a biodegradable polymer and a basic excipient.

2. The modified biodegradable microsphere of claim 1 wherein the drug comprises a protein or peptide.

3. The modified biodegradable microsphere of claim 2 wherein the protein is insulin.

4. The modified biodegradable microsphere of claim 1 wherein the biodegradable polymer comprises poly(L-lactic acid) or a copolymer thereof with D-lactic acid or glycolic acid.

5. The modified biodegradable microsphere of claim 1 wherein the basic excipient comprises a bicarbonate.

6. A method of improving the release profile of a drug encapsulated within a biodegradable microsphere comprising incorporating a basic excipient into a biodegradable polymer to form a microsphere and encapsulating the drug within the microsphere.

7. The method of claim 6 wherein the drug comprises a protein or peptide.

8. The method of claim 7 wherein the protein is insulin.

9. The method of claim 6 wherein the biodegradable polymer comprises poly(L-lactic acid) or a copolymer thereof with D-lactic acid or glycolic acid.

10. The method of claim 6 wherein the basic excipient comprises a bicarbonate.

11. A method of delivering a drug to a patient comprises encapsulating the drug in a biodegradable polymer microsphere comprising a biodegradable polymer and a basic excipient and administering the encapsulated drug to the patient.

12. The method of claim 11 wherein the encapsulated drug is administered parenterally.

13. The method of claim 11 wherein the drug comprises a protein or peptide.

14. The method of claim 13 wherein the protein is insulin.

15. The method of claim 11 wherein the biodegradable polymer comprises poly(L-lactic acid) or one of its copolymers with D-lactic acid or glycolic acid.

16. The method of claim 11 wherein the basic excipient comprises a bicarbonate.

Docket No.
RU-0174

Declaration and Power of Attorney For Patent Application

English Language Declaration

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name,

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

Modified Biodegradable Polyester Microspheres for Stabilizing and Improving the Release Profile of Drugs Encapsulated Within the Microspheres

the specification of which

(check one)

☐ is attached hereto.

☒ was filed on **30 May 2000** as United States Application No. or PCT International Application Number **PCT/US00/14875**

and was amended on _____
(if applicable)

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, Section 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, Section 119(a)-(d) or Section 365(b) of any foreign application(s) for patent or inventor's certificate, or Section 365(a) of any PCT International application which designated at least one country other than the United States, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate or PCT International application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application(s)			Priority Not Claimed
(Number)	(Country)	(Day/Month/Year Filed)	☐
(Number)	(Country)	(Day/Month/Year Filed)	☐
(Number)	(Country)	(Day/Month/Year Filed)	☐

I hereby claim the benefit under 35 U.S.C. Section 119(e) of any United States provisional application(s) listed below:

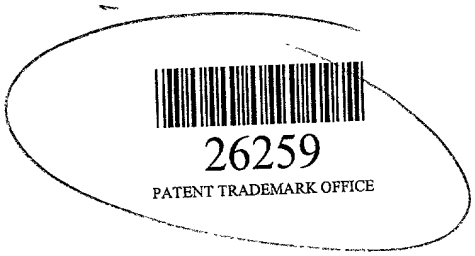
60/137,289	June 3, 1999
(Application Serial No.)	(Filing Date)
(Application Serial No.)	(Filing Date)
(Application Serial No.)	(Filing Date)

I hereby claim the benefit under 35 U. S. C. Section 120 of any United States application(s), or Section 365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of 35 U.S.C. Section 112, I acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, C. F. R., Section 1.56 which became available between the filing date of the prior application and the national or PCT International filing date of this application:

(Application Serial No.)	(Filing Date)	(Status) (patented, pending, abandoned)
(Application Serial No.)	(Filing Date)	(Status) (patented, pending, abandoned)
(Application Serial No.)	(Filing Date)	(Status) (patented, pending, abandoned)

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith. *(list name and registration number)*



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1.00

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Docket No.
RU-0174

Declaration and Power of Attorney For Patent Application

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Prior Foreign Application(s)			Priority Not Claimed
(Number)	(Country)	(Day/Month/Year Filed)	☐
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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith. *(list name and registration number)*

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PATENT TRADEMARK OFFICE

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